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# Lignans with Anti-Hepatitis B Virus Activities from *Phyllanthus niruri* L.

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**One new lignan, nirtetralin B, along with its two known stereoisomers were isolated from *Phyllanthus niruri* L. The structure of the new compound was determined by spectroscopy experiments and x-ray diffraction analysis. These lignans were assayed for anti-hepatitis B virus activities *in vitro*. Nirtetralin and nirtetralin A, B effectively suppressed the secretion of the HBV antigens in a dose-dependent manner with $IC_{50}$ values for HBsAg of 9.5 μM (nirtetralin A), 16.7 μM (nirtetralin B) and 97.2 μM (nirtetralin), $IC_{50}$ values for HBeAg of 17.4 μM (nirtetralin A), 69.3 μM (nirtetralin B) and 232.0 μM (nirtetralin), respectively. Copyright © 2011 John Wiley & Sons, Ltd.**

*Keywords: Phyllantus niruri* L.; lignans; nirtetralin B; anti-HBV.

Supporting information may be found in the online version of this article (Supplementary Material)

## INTRODUCTION

*Phyllanthus niruri* L. *(P. niruri)* distributes in tropical and subtropical regions. It has been thoroughly investigated for its significant activities of anti-hepatitis B virus (Blumberg *et al*., 1990; Lee *et al*., 1996; Thyagarajan *et al*., 1988). Previous studies on this plant have led to the discovery of 21 lignans, which are mainly phenyltetralin and dibenzylbutane types (Li *et al*., 2007), among them niranthin and nirtetralin suppressed effectively both HBsAg and HBeAg expression *in vitro* (Huang *et al*., 2003). As an investigation of new anti-HBV lignans from herbal sources, the chemical studies of *P. niruri* led to the isolation of a new lignan nirtetralin B (5R, 6S, 7R) – 5-(3′,4′-dimethoxyphenyl)-4-methoxy-6, 7-bis(methoxymethyl)-5, 6,7,8-tetrahydronaphtho [2, 3-d] [1″,3″] dioxole) (**1**) along with its two known stereoisomers neonirtetralin (renamed as nirtetralin A, 5S, 6S, 7R)-5- (3′,4′- dimethoxyphenyl)-4- methoxy-6,7- bis (methoxymethyl)-5,6,7,8-tetrahydronaphtho [2,3-d][1″,3″] dioxole (**2**) (Wei *et al*., 2002) and nirtetralin (5R, 6S,7S)-5-(3′,4′-dimethoxyphenyl)-4-methoxy-6,7-bis (methoxymethyl)-5, 6, 7, 8-tetrahydronaphtho [2, 3-d] [1″,3″] dioxole) (**3**) (Anjaneyulu *et al*., 1973). Lignans **1**, **2** and **3** belong to the phenyltetralin type. The study describes herein the isolation, structural elucidation of these compounds, as well as their anti-HBV (hepatitis B virus) and cytotoxic activities against HepG 2.2.15 cell line *in vitro*. Bioactive assays showed that three stereoisomers **1**, **2** and **3** effectively inhibited the secretion of the HBV antigens.

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## MATERIALS AND METHODS

**Plant material.** Herb material of *Phyllantthus niruri* L. was collected in Longan, Guangxi province, China, in November 2007, and identified by Dr Shifeng Ni, Northwest University, Xi an, China. A voucher specimen (2007-p-1) was deposited in the Department of Chemistry, Guangxi University for future reference.

**Methods.** Optical rotations were obtained from a Jasco 1010 polarimeter. The IR spectra were measured on a Thermo Nicolet Nexus 470 spectrophotometer. The UV spectra were measured on Shimadzu UV-2501PC spectrophotometers. The 1D and 2D NMR spectra were recorded on Bruker Avance 500 MHz spectrometers (500 MHz for $^{1}H$ and 125 MHz for $^{13}C$, respectively). The NOESY spectrum was recorded on Varian 600 MHz spectrometers. EI-MS was measured on a Shimadzu GCMS-QP5050A gas chromatograph mass spectrometer. HR-EI-MS was recorded on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (APEX IV, Bruker Co.). The x-ray crystallographic data were collected on a Rigaku R-Axis Rapid diffractometer equipped with Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods using SHELXL-97. Silica gels (200–300 mesh, Qingdao Marine Chemical Inc., China) were used for column chromatography. Aciclovir (ACV, purity > 99%, HPLC, Toronto Research Chemicals Inc. Canada) was used as a positive control in bioactive assays. Fractional eluates were monitored by TLC and then combined. Spots on TLC were visualized by using ultraviolet light (254 nm) and then sprayed with 5% $H_2SO_4$–MeOH solution followed by heating. Melting points were determined on an X-4 melting point apparatus (Beijing Taike Instrument Co. Ltd).

Inhibiting the secretion of the HBV antigens was used to evaluate the activities of samples with the ELISA

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method (enzyme-linked immunosorbent assays for qualitative detection of hepatitis B viruses in human serum or plasma samples) described in previous papers (Shin *et al*., 2005; Li *et al*., 2008). The anti-HBV activities of the test compounds were expressed as the 50% inhibitory concentration ($IC_{50}$) determined from the dose–response curve. The cytotoxicities of samples against the cell line were evaluated with the MTT colorimetric assay and expressed as half of the toxic dose ($TC_{50}$) described in previous reports (Korba *et al*., 1989; Wu *et al*., 1998). The *in vitro* inhibitory assays were performed on the human HBV transfected cell line HepG 2.2.15 (provided by The Institute of Medicinal Biotechnology of the Chinese Academy of Medical Sciences). Cultures of HepG 2.2.15 cell line were carried out according to the method described (Sells *et al*., 1987). The cells were cultured in modified Eagle's medium (MEM, Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (Hyclone Lab, USA), 0.03% L-glutamine (Beijing Solarbio Science and Technology Co., Ltd), G418 (Amresco, USA) 380 μg/mL, kanamycin (Amresco, USA) 50 U/mL, pH = 7.2 at 37 °C in a humidified incubator with 5% $CO_2$. The cells were seeded in 24-well tissue culture plates at approximately $1 \times 10^6$/mL and maintained at confluence for 48 h prior to the initiation of the treatment to allow the HBV DNA levels to stabilize. All compounds nirtetralin A (**1**, purity > 99%, $^1$H-NMR and $^{13}$C-NMR), nirtetralin B (**2**, purity > 99%, $^1$H-NMR and $^{13}$C-NMR) and nirtetralin (**3**, purity > 99%, $^1$H-NMR and $^{13}$C-NMR) used in this study were dissolved in DMSO and aciclovir (ACV, purity > 99%, HPLC) was used as the positive control. Cells were grown in medium containing 0.2% DMSO. The confluent HepG 2.2.15 cells were fed with the medium containing the indicated concentrations of test samples. The cell culture medium and HepG 2.2.15 cells were collected right before the first dose (day 0) and after 8 days of treatment. They were stored at −70 °C until analysis. The cell culture medium was used for the measurement of hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg).

**Extraction and isolation.** The air-dried whole plants of *P. niruri* (5 kg) were powdered and then extracted at room temperature with methanol (2 × 20 L) for 2 days. The methanol extracts were filtered and then evaporated to dryness under vacuum to give a residue 500 g. The residue (500 g) was suspended in water (3 L) and partitioned with petroleum ether (b.p. 60–90 °C, 5 × 2 L). The petroleum ether fraction (120 g) was chromatographed over a silica gel column (200–300 mesh, 15 × 100 cm, 2000 g) with petroleum ether-ethyl acetate as eluents (100:0 (10 L), 96:4 (10 L), 90:10 (7 L); 80:20 (7 L); 70:30 (7 L), 60:40 (5 L), 50:50 (5 L)). Eluates (500 mL per fraction) were examined by TLC and combined to afford eight fractions (Fr A (52.5 g), Fr B (15.6 g), Fr C (8.1 g), Fr D (5.5 g), Fr E (7.3 g), Fr F (2.8 g), Fr G (10.6 g) and Fr H (4.5 g)). Fr C (8.1 g) was chromatographed over a silica gel column (5 × 60 cm, 200–300 mesh, 200 g) eluted with petroleum ether-ethyl acetate (35:1, 3500 mL) solvent system to afford six subfractions (Frs 1–6) according to the TLC examinations. Subsequent separation of Fr 2 (0.35 g) by silica gel column chromatography (3 × 100 cm, 200–300 mesh, 70 g) using petroleum ether-ethyl acetate (35:1, 1000 mL) afforded a colorless oil. The colorless oil was finally purified on silica gel chromatography column (2 × 60 cm, 300–400 mesh, 20 g) eluted with petroleum ether-ethyl acetate (35:1, 1000 mL) to give compound **2** (148 mg). Separation of Fr 4 (2.8 g) on silica gel column chromatography (5 × 100 cm, 200–300 mesh, 250 g) using petroleum ether-ethyl acetate (30:1, 5000 mL) solvent system yielded a colorless crystal. The crystal was finally purified by crystallizing in methanol to afford compound **1 (**175 mg). Fraction 3 (0.15 g) was separated on a silica gel column (300–400 mesh, 3 × 100 cm, 70 g) eluted with petroleum ether-ethyl acetate (28:1, 1000 mL) to afford colorless crystal. The crystal was purified finally by crystallizing in methanol to afford compound **3** (75 mg). Compound **1** was obtained as a colorless crystal. Its molecular formula was determined as $C_{24}H_{30}O_7$ by NMR data (Table 1), EI-MS and HR-EI-MS (*m/z* 430.1987 [$M^+$]). Compound 1, a colorless crystal, $[\alpha]_D^{25}$ +7.5 (C = 8.76, $Me_2CO$). EI-MS *m/z*: 430 [$M^+$]. HR-EI-MS: *m/z* 430.1987 [$M^+$] (calc. for $C_{24}H_{30}O_7$, 430.1991). mp 81–83 °C (uncorr.). UV$\lambda_{max}$ (MeOH) nm (ε) 241 (10000), 281(5495). IR (KBr) cm$^{-1}$: 3056, 2927, 2869, 2833, 1624, 1516, 1462, 1252, 1135, 1108. NMR data: see Table 1. Crystal data: T = 153 (2) K, radiation (λ) Mo KR (0.71073 Å), θ = 27.48°, crystal size 0.54 × 0.52 × 0.37 mm, orthorhombic, space group P2 (1) 2(1) 2(1), a = 8.3570(2) Å, b = 16.4417(4) Å, c = 16.6870(4) Å, v = 2292.85(10) Å$^3$, Z = 4, $D_{calcd}$ = 1.247 m g/m$^3$, absorption coefficient, 0.091 mm$^{-1}$, F (000)) = 920.0, Reflections collected/unique, 22494/2970 [R (int) = 0.0140], Final R indices [I > 2σ (I)], R1 = 0.0302, wR2 = 0.0859, R indices (all data), R1 = 0.0307, wR2 = 0.0863, goodness of fit, 0.999. (CCDC 733444 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the CCDC, 12 Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk, or obtained as supporting information).

**Structural elucidation of compounds.** The UV absorption recorded at $\lambda_{max}$ 241 (ε = 10000) and 281 (ε = 5495) nm revealed that **1** is highly conjugated. The IR spectrum of **1** showed absorption bands (3056, 1624, 1516 cm$^{-1}$) for aryl groups. A close inspection of the NMR spectra of **1** by DEPT and HMQC experiments showed the presence of five oxygen-bearing carbon methyl groups (carbon signals at δ 56.0 (C-23), δ 56.1 (C-24), δ 58.9 (C-18), δ 59.0 (C-20), δ 59.2 (C-22)), four methylenes (carbon signals at δ 33.5 (C-4), δ 73.8 (C-17), δ 76.3 (C-19), δ 100.8 (C-21)) including oxygen-bearing carbons (C-17 and C-19) and one dioxygen-bearing carbon (C-21), four sp$^2$-hybridized methines in aromatic ring (carbon signals at δ 103.0 (C-6), δ 110.9 (C-13), δ 112.2 (C-16), δ 120.0 (C-12) ), three sp$^3$-hybrided methines (carbon signals at δ 37.1 (C-3), δ 41.5 (C-1), δ 45.43 (C-2)) and eight quaternary sp$^2$-hybrided carbons (signal at δ 124.9 (C-5), δ 132.1 (C-10), δ 135.7 (C-7), δ 139.9 (C-11), δ 142.0 (C-9), δ 147.0 (C-15), δ 147.6 (C-8), δ 148.7 (C-14)) including oxygen-bearing carbons (C-7, C-8, C-9, C-14, C15). The COSY of **1** revealed the presence of segments CH (H-1)-CH-(H-2)-CH (H-3)-$CH_2$ (H-4), CH (H-2)-$CH_2$ (H-17), CH (C-3)-$CH_2$ (H-19) and CH (H-12)-CH (H-13) (Fig. 1) on the basis of correlations between proton signals at δ 4.30 (H-1) and δ 2.01 (H-2); between proton signals δ 2.01 (H-2) and δ 1.82 (H-3); between proton signals at δ

**Table 1. $^{1}$H- and $^{13}$C-NMR data for compound 1 (in $CDCl_3$)**

| No. | $\delta_C$ [a] | $\delta_H$ (*J* in Hz) [a,b] | $^{1}$H-$^{1}$H COSY [c] | HMBC [d] | NOESY |
|---|---|---|---|---|---|
| 1 | 41.5 | 4.30(d, *J* = 6.16, 1H) | 2 | 3, 5, 10, 9, 11, 12, 16 | 12,17, 22 |
| 2 | 45.4 | 2.01(m,1H, *J* = 5.4,5.4,3.0, 4.8, 5.4) | 1, 3, 17 | | 4B, 17, 19, 12 |
| 3 | 37.1 | 1.82(m, 1H, *J* = 4.8, 6.6, 4.2, 3.6, 4.2, 6.6, 5.4 | 2, 4, 19 | | 4A |
| 4 | 33.5 | 2.73(1H, dd, *J* = 4.2, 3.6, 11.3, HA) | 4B | | 3, 6, 17, 19 |
| 4 | 33.5 | 2.58(1H, dd, *J* = 11.4, 12, HB) | 3, 4A | 5,6,10, 19, 2 | 2, 17, 19 |
| 5 | 124.9 | | | | |
| 6 | 103 | 6.42(s, 1H) | | 4, 5, 7, 8 | 4A |
| 7 | 135.7 | | | | |
| 8 | 147.6 | | | | |
| 9 | 142 | | | | |
| 10 | 132.1 | | | | |
| 11 | 139.9 | | | | |
| 12 | 120 | 6.56(1H, m, J12, 16 = 1.5, 1.4, J12, 13 = 8.2) | 13 | | 1, 2 |
| 13 | 110.9 | 6.72(1H, dd, *J* = 8.27, 1.47) | 12 | | 1, 2, 12 |
| 14 | 148.7 | | | | |
| 15 | 147 | | | | |
| 16 | 112.2 | 6.71(d, *J* = 1.5, 1H) | | 1,11,12, 14, 15 | |
| 17 | 73.8 | 3.39(m, *J* = 4.2, 4.8,4.2, 2H) | 2 | | 3, 1 |
| 18 | 58.9 | 3.30(s, 3H) | | 17 | |
| 19 | 76.3 | 3.35(m, *J* = 5.4, 4.4, 2H) | 3 | | 3, 2, 1 |
| 20 | 59 | 3.28(s, 3H) | | 19 | |
| 21 | 100.8 | 5.86(d, *J* = 5.82) | | 7, 8 | |
| 22 | 59.2 | 3.49(s, 3H) | | 9 | |
| 23 | 56 | 3.83(s, 3H) | | 14 | |
| 24 | 56.1 | 3.83(s, 3H) | | 15 | |

[a] $^{1}$H (500 and 600 MHz) and $^{13}$C (125 MHz) NMR spectral data measured in $CDCl_3$, values relative to internal TMS.
[b] Multiplicity of signals was on the base of DEPT (θ = 90° and θ = 135°) experiment, and was given in parentheses: s, singlet; d, doublet; br, broad; coupling constants (apparent splittings) are reported as numerical values in Hz.
[c] Signal correlating with $^{1}$H resonance.
[d] Signal correlating with $^{13}$C resonance.

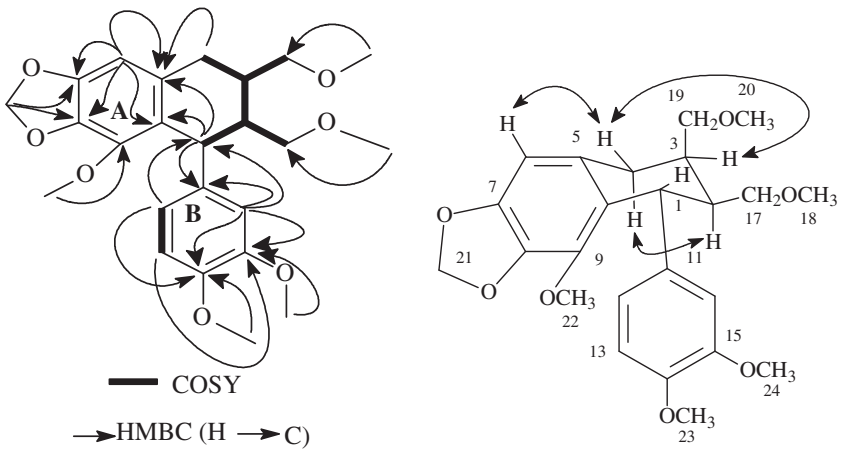


**Figure 1.** COSY, HMBC and NOESY correlations of **1**.

1.82 (H-3) and $\delta$ 2.58, 2.73 (H-4); between proton signals $\delta$ 2.01 (H-2) and 3.39 (H-17); between proton signals at $\delta$ 1.82 (H-3) and δ 3.35 (H-19); between proton signals at $\delta$ 6.56 (H-12) and $\delta$ 6.72 (H-13). The HMBC spectrum revealed significant correlations (Fig. 1, Table 1) between proton signal at $\delta$ 6.42 (H-6) and carbon signal at $\delta$ 33.5 (C-4), $\delta$124.9 (C-5), $\delta$ 135.7 (C-7), and $\delta$ 147.6 (C-8); between proton signal at $\delta$ 4.30 (H-1) and carbon signal at $\delta$ 132.1 (C-10); between proton signal at $\delta$ 2.60 (H-4B) and carbon signal at $\delta$ 103.0 (C-6), $\delta$ 124.9 (C-5), $\delta$ 132.1 (C-10); between proton signal at $\delta$ 3.30 (H-18) and carbon signal at $\delta$ 73.8 (C-17); between proton signal at $\delta$ 3.28 (H-20) and carbon signal at $\delta$ 76.3 (C-19; between proton signal at $\delta$ 5.86 (H-21) and carbon signal at $\delta$ 135.7 (C-7) and $\delta$147.6 (C-8); between proton signal at $\delta$ 3.49 (H-22) and carbon signal at $\delta$ 142.0 (C-9), that led to an establishment of a tetralin segment A (Fig. 1). Correlations between proton signal at $\delta$ 6.71 (H-16) and carbon signal at $\delta$ 139.9 (C-11), $\delta$ 120.0 (C-12),$\delta$ 148.7 (C-14) and

δ147.0 (C-15); between proton signal at δ 3.83 (H-23) and carbon signal at δ148.7 (C-14), proton signal at δ3.83 (H-24) and carbon signal at δ 147.0 (C-15) in HMBC led to an establishment of 3,4-dimethoxyl phenyl segment B (Fig. 1). Correlations between proton signal at δ 4.30 (H-1) and carbon signal at δ 139.9 (C-11), δ 120.0 (C-12) and δ 112.2 (C-16), between proton signal at δ 6.71 (H-16) and carbon signal at δ 41.5 (C-1), revealed that carbon signal at δ 41.5 (C-1) connected to phenyl groups A and B. On the base of COSY and HMBC correlations, the structure of **1** was established (Fig. 1). X-ray diffraction analysis of compound 1 (Fig. 2) showed its relative configuration and as an stereoisomer of nirtetralin (Anjaneyulu *et al*., 1973; Chuan *et al*., 2003). The optical data of compound 1 are different from both nirtetralin and nirtetralin A. The coupling constant $J = 6.14$ Hz of H-1 indicated a dihedral angle of H-C(1)-C(2)-H is about 145° (in *trans*) according to Karplus equation (Gutowsky *et al*., 1959), it showed that these two protons adopted axial orientations. Those correlations between H-4A and H-3, H-6 and H-A4 in NOESY indicated that H-3 and H-4A adopted equatorial orientation. A fact that coupling constants $J = 4.2$ Hz of H-3 and H-4A implied a 44° dihedral angle of H(A)-C(4)-C(3)-H confirmed H-3 and H-4A adopted equatorial orientation (in *cis*). Correlation between H-2 and H-4B in NOSEY indicated that H-2 adopted an axial orientation. Coupling constants $J = 5.4$ Hz of H-2 and H-3 indicated this two protons adopting a dihedral angle of 35°. So the structure of compound **1** was determined as (5R, 6S, 7R) – 5-(3′,4′-dimethoxyphenyl)-4-methoxy-6,7-bis(methoxymethyl)-5,6,7,8-tetrahydronaphtho [2, 3-d][1″, 3″] dioxole (Fig. 1, this name was nominated on the base of IUPAC), named nirtetralin **B**.

Compounds **2** and **3** were elucidated by comparing with reported data respectively as nirtetralin **A** (**2)** (5S, 6S, 7R)-5-(3′,4′- dimethoxyphenyl)-4-methoxy-6, 7-bis(methoxymethyl)-5,6,7,8-tetrahydronaphtho [2, 3-d] [1″, 3″] dioxole (Wei *et al*., 2002) and nirtetralin (**3)**, (5R, 6S, 7S)-5-(3′, 4′- dimethoxyphenyl)-4-methoxy-6, 7-bis(methoxymethyl)-5,6,7,8-tetrahydronaphtho [2, 3-d] [1″, 3″] dioxole (Anjaneyulu *et al*., 1973) by comparing their NMR with those in the literature and other physical data.

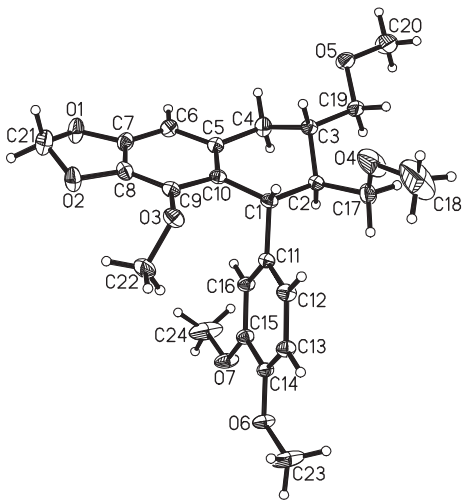


**Figure 2.** ORTEP drawing of **1**.

## RESULTS AND DISCUSSION

Cytotoxic activity and activities against the HepG 2.2.15 cell line of the lignans were tested. The results showed that the cytotoxic concentration required to cause 50% death of the HepG 2.2.15 cell ($TC_{50}$) was 69.8 μM (nirtetralin **A**), 1162.8 μM (nirtetralin **B**) and 1162.8 μM (nirtetralin). Maximum nontoxic concentrations ($TC_0$) for HBV were 17.4 μM (nirtetralin **A**), 72.7 μM (nirtetralin **B**) and 581.4 μM (nirtetralin) (Table 2). Nirtetralin, nirtetralin **A** and **B** effectively suppressed the secretion of the HBV antigens in a dose-dependent manner with $IC_{50}$ values for HBsAg of 9.5 μM (nirtetralin **A**), 16.7 μM (nirtetralin **B**) and 97.2 μM (nirtetralin), $IC_{50}$ values for HBeAg of 17.4 μM (nirtetralin **A**), 69.3 μM (nirtetralin **B**) and 232.1 μM (nirtetralin) at day 8. The inhibition ratios for HBV of the nirtetralin and nirtetralin B were highly significant comparing with the control ACV (Table 3). The results showed that nirtetralin and nirtetralin B are more effective than the control ACV in suppressing the secretion of the HBV antigens (ACV, 1000 μM, inhibition ratio 16.15% for HBsAg and 26.15% for HBeAg) (Cao and Tao, 2001). That nirtetralin and nirtetralin B effectively suppressed the secretion of HBsAg and HBeAg from HepG 2.2.15 cell line indicated that they decreased HBV concentration and inhibited the replication of hepatitis virus B. The activities for suppressing both HBsAg and HBeAg expression of nirtetralin were confirmed herein (Huang *et al*., 2003). Another lignan, niranthin, from this plant showed a more significant anti-HBV activity (Huang *et al*., 2003). Niranthin and the three lignans possess the same part of fragment, 4-methoxy-6-(3-methoxypropyl)benzo[d] [1′, 3′]dioxole, in their molecular structures. Meanwhile another fragment, 1, 2-dimethoxy-4-(3-methoxypropyl) benzene, appeared in niranthin, the three lignans and phyllanthin and hypophyllanthin (Chuan *et al*., 2003). Phyllanthin and hypophyllanthin, which lack the

**Table 2. Cytotoxic activity of lignans against HepG 2.2.15 cell line**

| | Concentration (μM) | | | | | | | | | | TC50 (μM) | TC0 (μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1162.8 | 581.4 | 290.7 | 145.4 | 72.7 | 69.8 | 36.3 | 34.9 | 17.4 | 8.7 | | |
| [a]CPE (%) of Nirtetralin | 50 | 0 | 0 | 0 | 0 | | 0 | | | | 1162.8 | 581.4 |
| [a]CPE (%) of Nirtetralin B | 50 | 25 | 25 | 8.3 | 0 | | 0 | | | | 1162.8 | 72.7 |
| [a]CPE (%) of nirtetralin A | | | | | | 50 | | 17 | 0 | 0 | 69.8 | 17.4 |

[a]CPE, cytophatic effect.

**Table 3. Anti-HBV activities of lignans**

| Sample | Conc. (μM) | Inhibition ratio of HBsAg (%) | Inhibition ratio of HBeAg (%) | $IC_{50}$ for suppress secretion of HBsAg (μM) | $IC_{50}$ for suppress secretion of HBeAg (μM) |
|---|---|---|---|---|---|
| Nirtetralin A | 17.4 | 64.3 | 14.3 | 9.5 | 17.4 |
| Nirtetralin B | 69.8 | 91.3 | 32.6 | 16.7 | 69.3 |
| Nirtetralin | 465.1 | 93.4 | 91.4 | 97.2 | 232.1 |
| Acyclovir | 1387.6 | 85 | 95.2 | | |

fragment 4-methoxy-6-(3-methoxypropyl) benzo[d][1′,3′] dioxole in their structures, did not show anti-HBV activities in our investigations and other reports (Huang *et al*., 2003). Thus a conclusion was proposed that the fragment 4-methoxy-6-(3-methoxypropyl)benzo[d][1′,3′] dioxole is key for anti-HBV activities in these lignans from *Phyllanthus niruri* L.

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## Conflict of Interest

The authors have declared that there is no conflict of interest.

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